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(54) **Removal of process chemicals from labile biological mixtures by hydrophobic interaction chromatography.**

(57) **A method of removing lipid soluble chemicals from a biological material containing the lipid soluble chemicals comprising subjecting the biological material containing the lipid soluble chemicals to hydrophobic interaction chromatography, preferably using a resin comprising octadecyl chain coupled to a silica matrix.**

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REMOVAL OF PROCESS CHEMICALS FROM LABILE BIOLOGICAL MIXTURES BY HYDROPHOBIC INTERACTION CHROMATOGRAPHY

BACKGROUND OF THE INVENTION

5 Field of the Invention

The present invention concerns the removal of process chemicals from labile biological mixtures by hydrophobic interaction chromatography. More particularly, the present invention concerns the removal of lipid soluble process chemicals, for example, chemicals used in the inactivation of viruses in plasma, cryoprecipitates and plasma-derived therapeutic preparations.

Background Information

15 Blood can contain each of several different viruses including but not limited to hepatitis B virus (HBV), non-A, non-B hepatitis virus (NANBHV), cytomegalovirus, and immunodeficiency viruses. It is highly desirable to inactivate these viruses in the course of preparing blood products and prior to the therapeutic application of blood and blood fractions. Both physical (e.g., heat, irradiation) and chemical (e.g., aldehydes, organic solvents, detergents, etc.) methods have been used to inactivate viruses such as HBV in mammalian blood and blood fractions. Inactivation renders a virus non-infectious and non-pathogenic.

20 Numerous attempts have been made to inactivate viruses such as hepatitis B virus (HBV) in mammalian, especially human, blood plasma. It is the practice in some countries to effect inactivation of the hepatitis B virus in the blood plasma by bringing the plasma into contact with a viral inactivating agent which crosslinks the proteinaceous portions of hepatitis B virus or which interacts with the nucleic acid of the virus. For instance, it is known that hepatitis B virus is inactivated by contact with an aldehyde, such as formaldehyde.

Among the procedures for inactivating viruses are the use of lipid solvents with the addition of surface active agents (A. M. Prince, B. Horowitz, B. Brotman et al, "Inactivation of Hepatitis B and Hutchinson Strain non-A, non-B Hepatitis Viruses by Exposure to Tween 80 and Ether", *Vox Sang.*, (1984), 46, 36-43; A. M. Prince, B. Horowitz and B. Brotman, "Sterilisation of Hepatitis and HTLV-III Viruses By Exposure to Tri(n-Butyl)Phosphate and Sodium Cholate", *The Lancet*, 706-710, March 29, 1986), and lipid solvents without the additive of surface active agents (S. M. Feinstone, K. B. Mihalik, T. Kamimura et al, "Inactivation of Hepatitis B Virus and non-A, non-B Hepatitis by Chloroform, *Infect. Immunol.*, (1983), 41, 816-821; D. W. Bradley, J. E. Maynard, H. Popper et al, "Posttransfusion non-A, non-B Hepatitis: Physicochemical Properties of Two Distinct Agents", *J. Infect. Dis.*, (1983), 148, 254-265).

35 U.S. patents 4,481,189 and 4,540,573, the entire contents of which are incorporated by reference herein, describe the use of organic solvent/detergent pairs to reduce the infectivity of hepatitis viruses and certain other viruses contained in or added to plasma and plasma products by several orders of magnitude. An example of such a pair is tri-n-butyl phosphate, an organic solvent, and TRITO X-100, a non-ionic detergent.

40 Solvent/detergent treatment under appropriate conditions of temperature and contact time effectively disassembles viruses that have envelope proteins associated with lipid, while having negligible effect on the molecular conformations and biological activities of sensitive blood plasma proteins. The independent effects of organic solvents and detergents in disassembling and attenuating viruses can be facilitated by the presence of both. Merocyanine, beta-propiolactone and cis-platin are among other agents that are applied to blood to inactivate viruses, though by mechanisms other than envelope disruption.

Removal of organic solvents, detergents and other virus-inactivating agents from biological products is necessary if a particular substance is not well tolerated by humans or other biological systems in which it is to be used, e.g., in tissue cultures. In the preparation of purified plasma proteins such as coagulation factor VIII or mixtures of selected proteins, the separation of the desired product from the virus-inactivating agents is often facilitated by a purification process. Thus, precipitation of the desired protein or positive adsorption by an immobilized product-specific ligand can often reduce the level of residual agents to "tolerable" levels by allowing the majority to be washed away.

Other methods used to achieve removal of lipid/detergent micelles from membrane-protein complexes

may be applicable to removal of the same from plasma products and other biologic products. These have been based on differences in size, buoyant density, charge, binding affinity, phase partitioning and solvent partitioning (A. Helenius and K. Sinous, "Solubilization of Membranes by Detergents", Biochem. Biophys. ACTA, (1975), 415, 29-79).

5 In the instance of whole blood plasma, blood serum, cryodepleted plasma or cryoprecipitate for direct use in transfusion, implementation of the organic solvent/ detergent method of virus sterilization has not occurred. Because preparation of these materials does not involve steps for fractionation, purification, or refinement, there is no convenient opportunity to effect removal of the inactivating agents by the methods suggested above. An efficient removal method is further constrained by the necessity of leaving the
10 composition and biological activity of the preparation substantially intact.

Another difficulty in preparing virus sterilized plasma and cryoprecipitate is in filtering the plasma after treatment to maintain bacterial sterility without loss of labile proteins and biological activity.

Thus, because virus sterilization techniques have not been applied to whole blood plasma, etc., virus infectivity upon infusion remains, estimated at 0.05% for hepatitis B and 3% for non-A, non-B hepatitis
15 transmission.

Exogenous chemicals are frequently added to biological mixtures to stimulate synthesis, inactivate viruses contained therein and to stabilize or purify desired components present in the mixture. It is desirable to remove these chemicals without otherwise affecting the structure and function of the desired components. For example, the synthesis of certain desired biological products can be induced or enhanced in cell
20 cultures by introduction of phorbol esters into the culture fluid. For example, mezerein may be used to induce gamma interferon production by cultured leukocytes (Y. K. Yip, R. H. L. Pang, J. O. Oppenheim, M. S. Nashbar, D. Henriksen, T. Zerebecky-Eckhardt, J. Vilcek, "Stimulation of Human Gamma Interferon Production by Diterpene Esters", Infect. and Immun., (1981) 131-139) or to augment secretion of tumor necrosis factor by cells that product it (B. D. williamson, E. A. Carswell, B. Y. Rubin, J. S. Prendergast, H. J. Old, "Human Tumor Necrosis Factor Produced by Human B-cells Lines: Synergistic Cytotoxic Interaction with
25 Human Interferon", Proc. Natl. Acad. Sci., USA, (1983), 80, 5397-5401).

Before use in man, phorbol esters must be removed from lymphokine preparations because of the carcinogenic properties of these compounds. Heretofore, phorbol esters have been removed by precipitation, chromatographic, or molecular exclusion processes, (B. Y. Rubin, S. L. Anderson, S. A. Sullivan, B. D. Williamson, E. A. Carswell, L. J. Old, "Purification and Characterization of a Human Tumor Necrosis Factor from the LukII Cell Line", Proc. Natl. Acad. Sci., USA, (1985), 82, 6637-6641).
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A method for removal of TNBP and other lipid soluble process chemicals from complex biological mixtures by extraction with vegetable oils, e.g., soy bean oil, is described in European Patent Application 87 103 212, filed March 17, 1987 (USP 4 789 545) This method does not remove most detergents.

35 A similar method for removal of TNBP, detergents and other lipid soluble chemicals by extraction with long-chain alcohols or halogenated esters is described in pending European Patent Application 88 121 582, filed December 23, 1988 (US patent application 07/139,502) Shortcomings of this method are that expensive and/or noxious chemicals are required and that additional steps must be taken to reduce to tolerable levels any residual extraction agents. An alternative method that efficiently removed TNBP and
40 detergents and did not present these problems would be desirable.

Hydrophobic interaction chromatography (HIC) is a commonly used tool in the biochemists' arsenal of molecular separation techniques. A description of the principles of HIC is given in S. Hjerten, "Some General Aspects of Hydrophobic Interaction Chromatography", J. of Chromatography, (1973), 87, 325-331. Briefly, a lipid-like moiety such as an alkyl chain coupled to an inert matrix is used to partition molecules
45 containing similar hydrophobic domains from aqueous solutions by virtue of their mutual affinity. The alkyl chain may range from two to twenty-four or more carbons in length and may be linear or branched and may contain or terminate in other hydrophobic groups such as a phenyl ring. Increasing chain length results in media with greater hydrophobic character.

In practice, the strength of the hydrophobic interactions is also influenced by the ionic strength, pH and
50 polarity of the solvent. For example, a high concentration (i.e., 4 molar) of ammonium sulphate in the solvent would promote hydrophobic interaction and, hence, binding between the resin and the hydrophobic domains of the solute proteins. Following absorption, the proteins can be eluted from the resin by using a buffer with lower ionic strength, chaotropic ions, and/or polarity-lowering additives, such as ethylene glycol or detergents. In addition to resins that react strictly through hydrophobicity, there are materials that work
55 via a combination of hydrophobic and ionic interactions such as amino-hexyl Sepharose (LKB/Pharmacia, Piscataway, N.J.) which incorporates an amino group at the end of a six carbon alkyl chain as the active function. Among the many plasma proteins that have been purified by these methods are albumin, transferrin, thyroglobulin, lactic dehydrogenase, beta lipoproteins, coagulation factors and immunoglobulins.

The inert matrix to which the hydrophobic groups are bound in the preparation of HIC media may be comprised of a polysaccharide, such as agarose or silica or other polymers. Agarose based media are relatively soft and, in a typical chromatography system, require slow flow rates and result in lengthy separation procedures. Silica based media, being incompressible, are typically employed in high pressure chromatography systems, but may be used in low pressure systems at relatively high flow rates.

Though generally used for the isolation of proteins, hormones, and other biological molecules from complex biological mixtures, HIC has also been used to remove detergent from certain biological preparations where the detergent was used to dissociate membranes. For example, C18 HIC medium in a cartridge (Sep-Pak, Waters Associates, Milford, M.A.), as well as an ion exchange column was used to remove TRITON X-100, protein, salts and other interfering compounds from rat brain homogenates in order to facilitate the measurement of inositol in the tissue extract (S.E. Laursen, H.R. Knull and J.K. Belknap, "Sample Preparation for Inositol Measurement: Sep-Pak C18 Use in Detergent Removal", Analytical Biochemistry, 153, 387-390 (1986)).

SUMMARY OF THE INVENTION

It has now been discovered, quite surprisingly, that HIC, normally performed under stringent conditions, such as high salt concentration in the loading solvent for the isolation of proteins, hormones and other substances from biological preparations, can be used under mild conditions - that is, at the native concentrations of proteins, salts and other physiologic constituents in biological fluids - to extract organic solvents and detergents added to these fluids for the purpose of inactivating viral contaminants.

It was further surprising to find that certain constituents of blood plasma, cryoprecipitate, etc., such as coagulation factors and other enzymes - proteins particularly sensitive to denaturation or inactivation or adsorption by hydrophobic media - were present at high concentration and biological activity following removal of virus inactivating agents by the present invention.

Also surprising was the discovery that when long chain alcohols, particularly 2-octanol or a halogenated ether, particularly isoflurane, or a combination of the two were used in purification of biological materials to extract virus inactivating agents, residuals of these compounds could be reduced to trace levels by application of HIC.

It is an object of the present invention to remove virus inactivating solvents and/or detergents and/or other process chemicals, such as phorbol esters, from biological materials, without destroying the properties of the desired components. This object and other objects are provided by the present invention wherein lipid soluble process chemicals, e.g., virus inactivating solvents and/or certain detergents and/or phorbol esters, are removed from biological materials, e.g., labile biological materials, by passing such biological materials containing such lipid soluble process chemicals through a hydrophobic interaction chromatography (HIC) column having as a packing a resin of C-6 to C-24 chains. Preferably the hydrophobic interaction chromatography is employed using octadecyl (C-18) chains coupled to a silica matrix support. The method of the present invention is particularly useful in removing tri-n-butyl phosphate (TNBP) and "TRITON X" detergent from complex biological mixtures such as plasma, cryoprecipitate, or AHF concentrate with little or no loss of coagulation factor activity and minimal change in protein composition.

In the present inventive method, the biological material substantially retains its activity. Furthermore, little or no biological material is absorbed during the passing through the column.

The present invention concerns methods for removing virus attenuating solvents from biological materials to which such solvents have been added. The present invention also concerns removal of certain virus attenuating detergents from biological materials to which such detergents have been added together with or without solvents.

The present invention still further concerns methods for removing other virucidal agents from biological materials.

The present invention further concerns methods for removing process chemicals added as stabilizers to biological materials.

The present invention also further concerns methods for removing process chemicals used in the purification of biological materials.

The present invention additionally concerns methods for removing naturally occurring lipids and other endogenous or exogenous, e.g., "TRITON X-45" and TNBP, lipid soluble compounds from biological materials, such removal resulting in improved properties of said materials, e.g., filterability, stability, or rendering virus sterilization reagents more effective.

Accordingly, the present invention concerns a method of improving the filterability and/or stability of biological fluids by removing exogenous or endogenous lipid soluble compounds from a biological material containing lipid soluble compounds, the method comprising passing a biological material containing lipid soluble compounds through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.

The present invention also concerns methods for the removal of chemical inducers such as lymphokine inducing phorbol esters (i.e., inducers of lymphokine synthesis) from biological materials.

The present invention further concerns the removal of process agents added to blood cells, without disruption of the cells.

DETAILED DESCRIPTION OF THE INVENTION

Blood is made up of solids (cells, i.e., erythrocytes, leucocytes, and thrombocytes) and liquid (plasma). The cells contain potentially valuable substances such as hemoglobin, and they can be induced to make other potentially valuable substances such as interferons, growth factors, and other biological response modifiers. The plasma is composed mainly of water, salts, lipids and proteins. The proteins are divided into groups called fibrinogen, serum globulins and serum albumin. Typical antibodies (immune globulins) found in human blood plasma include those directed against infectious hepatitis, influenza H, etc.

Cells found in blood include red cells, various types of leukocytes or white cells, and platelets. Fractionation of cell types typically utilizes centrifugation, but may involve other forms of differential sedimentation through addition of rouleaux enhancing agents such as hydroxyethyl starch, separations based on immunological specificity, etc.

Proteins found in human plasma include prealbumin, retinol-binding protein, albumin, alpha-globulins, beta-globulins, gamma-globulins (immune serum globulins), the coagulation proteins (antithrombin III, prothrombin, plasminogen, antihemophilic factor (factor VIII), fibrin-stabilizing factor-factor XIII, fibrinogen), immunoglobins (immunoglobulins G, A, M, D, and E), and the complement components. There are currently more than 100 plasma proteins that have been described. A comprehensive listing can be found in "The Plasma Proteins", ed. Putnam, F.W., Academic Press, New York (1975).

Proteins found in the blood cell fraction include hemoglobin, fibronectin, fibrinogen, enzymes of carbohydrate and protein metabolism, platelet derived growth factor etc. In addition, the synthesis of other proteins can be induced, such as interferons and growth factors.

A comprehensive list of inducible leukocyte proteins can be found in Stanley Cohen, Edgar Pick, J.J. Oppenheim, "Biology of the Lymphokines", Academic Press, N.Y. (1979).

Blood plasma fractionation generally involves the use of organic solvents such as ethanol, ether and polyethylene glycol at low temperatures and at controlled pH values to effect precipitation of a particular fraction containing one or more plasma proteins. The resultant supernatant can itself then be precipitated and so on until the desired degree of fractionation is attained. More recently, separations are based on chromatographic processes. An excellent survey of blood fractionation appears in Kirk-Othmer's Encyclopedia of Chemical Technology, Third Edition, Interscience Publishers, Volume 4, pages 25 to 62.

The major components of a cold ethanol fractionation are as follows:

Fraction	Proteins
I	fibrinogen; cold insoluble globulin; factor VIII; properdin
II and III	IgG; IgM; IgA; fibrinogen; beta-lipo- protein; prothrombin; plasminogen; plasmin inhibitor; factor V; factor VII; factor IX; factor X; thrombin; antithrombin; isoagglutinins; cerulo- plasmin; complement C'1, C'3
IV-1	alpha ₁ -lipoprotein, ceruloplasmin; plasmin-inhibitor; factor IX; peptidase; alpha-and-beta-globulins
IV-4	transferrin; thyroxine binding globulin; serum esterase; alpha ₁ -lipoprotein; albumin; alkaline phosphatase
V	albumin; alpha-globulin
VI	alpha ₁ -acid glycoprotein; albumin

The above fractionation scheme can serve as a basis for further fractionations. Fraction II and III, for example, can be further fractionated to obtain immune serum globulin (ISG).

Another fractionation scheme involves use of frozen plasma which is thawed into a cryoprecipitate containing AHF (antihemophilic factor) and fibronectin and a cryosupernatant. The cryoprecipitate is then fractionated into fibronectin and AHF.

The methods of the present invention are applicable to biological materials including blood cells, blood plasma, blood fractions thereof, and blood proteins (such as those discussed hereinabove, cryoprecipitate, cryodepleted serum and more generally to biological cells and fluids, e.g., normal cells, cancer cells, exudate from cancer cells grown in culture, exudate from normal cells grown in culture, cells from hybridomas, products of gene splicing, plant cell concentrates, plant cell suspensions, extracts of animal tissue, extracts of plant tissue and microorganisms).

Non-limiting examples of organic long chain alcohols for use in the present invention include hexanol, heptanol, 1-octanol, 2-octanol, 1-nonanol, 1-decanol and undecanol.

Non-limiting examples of halogenated (e.g., containing fluorine, chlorine, iodine and/or bromine) hydrocarbons for use in the present invention include 1,2,2-trifluoroethane, "ETHRANE" (enflurane; 2-chloro-1,1,2-trifluoroethyl difluoromethyl ether), "FORANE" (isoflurane; 1-chloro-2,2,2-trifluoroethyl difluoromethyl ether). Preferred halogenated hydrocarbons according to the present invention contain fluorine, chlorine and ether.

The typical solvents removed by the present invention are liquid at the temperature for use, immiscible with the aqueous solutions being extracted, non-denaturing to proteins and to cells under the conditions of use, easily removed, non-explosive, and non-toxic in the quantities remaining in the biological solution under the conditions of use.

Di- or trialkylphosphates, detergents and surfactants for removal by the process of the present invention are described in U.S. Patents 4,540,573 and 4,481,189.

Ranges for solvent and detergent encountered in treated biological materials to be subjected to HIC according to the present invention are as follows:

solvent : 1000-20,000 ppm

detergent : 1000-10,000 ppm.

The present invention is particularly directed, inter alia, to producing a protein-containing composition such as blood plasma, cryoprecipitates, blood plasma fractions, etc., which is substantially free of infectious virus, yet which retains a substantial amount of enzymatically or biologically active (undenatured) protein

and from which process chemicals have been removed so that the resultant composition has no more than physiologically acceptable levels of such process chemicals.

Biological fluids for use according to the present invention include blood plasma, blood plasma fractions, precipitates from blood fractionation and supernatants from blood fractionation. Also contemplated is the treatment of concentrates of whole blood cells, red cells, white cells (leukocytes), platelets, platelet rich plasma, platelet poor plasma, and concentrates of granulocytes, monocytes, or lymphocytes or other cells capable of producing interferon, tumor necrosis factor (TNF), or other immune modulators or lymphokines, or the media separated from such concentrates or suspension.

According to the present invention, there is contemplated the preparation of a protein-containing composition, particularly whole blood plasma or whole blood serum having an extent of inactivation of virus greater than 6 logs of virus, such as AIDS virus (HIV I), hepatitis B virus and non-A non-B hepatitis virus, having a retention of functional activity for particularly biologically active proteins of at least 45%, preferably at least 75%, more preferably at least 85%, even more preferably at least 85% and most preferably 98% to 100%, and having no more than physiologically acceptable levels of lipid soluble process chemicals.

Coagulation factor activity is retained at more than 60% of its original level and preferably more than 75 to 85%, and most preferably at more than 90 to 98% of its original level.

The (virus sterilized) whole blood plasma, blood serum, cryoprecipitate or cryodepleted plasma according to the present invention can be transfused directly into a patient, e.g., mammal, e.g., human. Alternatively, the (virus sterilized) whole blood plasma, blood serum, cryodepleted plasma or cryoprecipitate according to the present invention can be fractionated to prepare purified plasma protein derivatives (such derivatives can be transfused directly into a patient, e.g., a human patient).

The whole blood plasma or blood serum according to the present invention can also be used in cell cultures and as a quality control reagent.

Furthermore, non-blood sources including, for example, normal (noncancerous) or cancer cells, exudate from cancer or normal cells grown in culture, hybridomas and products from gene splicing, plant cell concentrates or suspensions, extracts of animal or plant tissues, or microorganisms can be used as the biological fluid in the present invention.

The process of the present invention differs from other applications of HIC in that materials and conditions are employed that minimize adsorption and separation of proteins and maximize the removal of lipid-soluble process chemicals as described.

The preferred resin for use in the present invention is Bulk C-18 packing from Waters, Inc. having a particle size of 55-105 microns and a porosity of 120 Angstroms. The active function is an eighteen carbon linear chain coupled to a silica matrix. The uncoupled sites on the matrix are blocked with dimethylsilane.

The capacity of this material for binding TRITON X-100 detergent is approximately 160 mg (0.25 millimoles) of detergent per gram of dry resin. Other C-18 resins from Waters such as Bondapack C-18 or Megabond C-18 of their equivalents from other manufacturers provide similar capacity. The use of a silica matrix permits the extraction process to occur at higher flow rates and at higher pressures than are obtainable with more compressible chromatography media. Typically, columns can be operated at a flow rate of 125 to 175 ml/cm²/hr or higher compared to 25 to 50 ml/cm²/hr for an agarose-based resin.

In practice, resin is preferably packed into a stainless steel or glass chromatography column. The column volume should preferably be at least 1/8 of the volume of the material to be loaded if the "TRITON" concentration in the material is 1% (w/v). The resin is activated by washing with isopropanol and then with water. Ethanol and acetonitrile are suitable organic phases for activation and regeneration of the resins. Before loading the column is preferably equilibrated with saline or an appropriate buffer.

The temperature range for the process is preferably from 4° to 37° C and most preferably 20° C to 25° C.

No more than 15 weight % of the biological material is adsorbed on the column and preferably no more than 5 to 10% is adsorbed. Most preferably, no more than 2 to 5% is adsorbed on the column.

The column is preferably cleaned and regenerated after absorbing the virus-inactivating agents by washing with water and increasing concentrations of ethanol or isopropanol from 15% to 100% and then water again. If ethanol is used, a column volume of 100% isopropanol should also preferably be used afterward.

Material to be processed according to the invention includes, for example, plasma, cryoprecipitate, AHF concentrate, immune globulin and Prothrombin Complex containing TNBP and "TRITON X-100". Other complex protein mixtures such as vaccines, coagulation factors, serum, etc. can be processed as described herein.

The present invention will now be described with reference to the following non-limiting examples.

EXAMPLES5 Example 1: Preparation of Virus-Sterilized Plasma

Six units of fresh frozen plasma were thawed and pooled. TNBP and TRITON X-100 were added to a concentration of 1% (w/v) and the solution was incubated for 4 hours at 37°C with gentle agitation. Following the incubation, the material was clarified, if necessary, by centrifugation at 10,000 x g. The plasma was then passed through a column containing 60 g of Bulk C-18 media (Waters, Inc., Milford, MA.) which had previously been washed with several column volumes of isopropanol followed by sterile saline. The flow rate through the column was approximately 150 ml/cm² per hour and the operating temperature was 23-25°C. After processing the plasma, the column was regenerated by washing with saline followed by gradient of 15 to 95% ethanol, followed in turn by 100% isopropanol. Table 1, below, illustrates the effectiveness of TNBP and TRITON removal and the recovery of coagulation factors V and VIII in eight batches of plasma processed as described. The activated partial thromboplastin time (APTT) is a comprehensive measure of clotting factor activity. The results in Table 1 indicate that 80 to 95% of clotting factor activity is retained. Table II shows the minor differences that exist in a pool of plasma before and after treatment as described with regard to a number of protein fractions, enzymes, and other constituents. In general, the levels of all of the constituents measured are within the normal physiological range. However, the lipid content is lower, resulting in increased filterability.

Table 1.

Summary of Virus Sterilized Plasma Preparations									
BATCH	1	2	3	4	5	6	7	8	AVG.
TNBP (ppm)	5.0	1.6	7.7	1.4	2.1	0.8	1.5	2.7	2.85
TRITON (ppm)	4.0	8.0	8.0	1.0	5.0	8.0	2.0	1.5	4.69
FACTOR VIII (units/ml)									
starting pool	0.96	1.08	1.13	1.23	1.13	0.99	1.12	1.34	1.12
after treatment	1.01	1.00	0.76	0.95	0.85	0.89	0.96	0.83	0.91
FACTOR V (units/ml)									
starting pool	1.05	0.88	1.08	1.04	1.18	1.11	1.04	0.78	1.02
after treatment	0.96	0.89	0.66	0.81	1.03	1.01	0.85	0.70	0.86
APTT (seconds)									
starting pool	31.2	30.5	29.3	29.2	29.5	29.5	28.5	29.2	29.6
after treatment	31.8	31.5	nd	30.8	38.3	nd	29.2	32.1	32.3
nd = no data									

Table 2

Some Characteristics of Plasma Before and After Virus-Sterilization		
	UNTREATED	TREATED
TOTAL PROTEIN g/dl	7.3	6.5
ALBUMIN g/dl	4.2	4.2
IgG mg/dl	1310	1200
IgA mg/dl	249	192
IgM mg/dl	183	90
C3 mg/dl	79	62
C4 mg/dl	33.4	22.1
HAPTOGLOBIN mg/dl	130	109
TRIGLYCERIDES mg/dl	176	104
CHOLESTEROL mg/dl	195	93
CALCIUM mg/dl	8	8.1
PHOSPHORUS mg/dl	12	12
IRON μ g/dl	182	199
BUN mg/dl	17	15
URIC ACID mg/dl	4.9	4.5
TOTAL BILIRUBIN mg/dl	0.3	0.3
CREATININE mg/dl	1.2	0.8
LDH units/ml	173	181
ALT units/ml	23	22
GGT units/ml	19	19
AMYLASE units/ml	32	30
ALK PHOS units/ml	100	52
SGOT units/ml	39	40
BUN = blood urea nitrogen ALT = alanine amino transferase GGT = gamma-glutamyl transpeptidase ALK PHOS = alkaline phosphatase SGOT = serum glutamic oxaloacetic transaminase LDH = lactic dehydrogenase		

Example 2: Removal of TNBP and "TRITON" from preparations of several products:

Each product was inactivated by incubation with TNBP (1% for plasma and cryo, 0.3% for other products) and 1% "TRITON X-100" for at least 3 hours. Material was clarified, if necessary, by centrifugation at 10,000 x g before loading on column. The column contained 12 g of Bulk C-18 packing (Waters, Inc.) which had been activated with several column volumes of organic phase (either isopropanol, acetonitrile, or ethanol) and then washed with several volumes of distilled water prior to loading of sample. In Table 3 below, recovery refers to the yield on the column step.

Table 3

Material Processed	Triton (ppm)	TNBP (ppm)	Recovery (%)	measured by
plasma-forane extr.	5.4	1.0	90	APPT
AHF concentrate	2.5	3.3	83	AHF activity
ISG	12.2	2.3	99	Total protein
Cryoprecipitate	7.0	0.6	86	AHF activity
extr. = plasma was extracted with forane prior to loading on the C ₁₈ column				
ISG = immune serum globulin				

It will be appreciated that the present specification and claims are set forth by way of illustration and not limitation and that various modifications and changes may be made without departing from the spirit and scope of the present invention.

Claims

1. A method of removing lipid soluble process chemicals from a biological material containing said lipid soluble chemicals, the method comprising passing said biological material containing said lipid soluble chemicals through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.

2. A method according to claim 1, wherein the resin comprises an active function and a support and wherein the active function comprises octadecyl chains and the support comprises a silica matrix, wherein the silica matrix preferably has uncoupled sites which are blocked with dimethylsilane.

3. A method according to claims 1 or 2, wherein the resin is activated with an organic solvent selected from the group consisting of isopropanol, ethanol and acetonitrile.

4. A method according to anyone of claims 1 to 3, wherein the column is operated at a flowrate of 125 to 175 ml/cm²/hour.

5. A method according to anyone of claims 1 to 4, wherein the method is conducted at a temperature of 4° C to 37° C, preferably from 20° C to 25° C.

6. A method according to anyone of claims 1 to 5, wherein the lipid soluble process chemicals are selected from the group consisting of an organic solvent, a detergent, a long chain alcohol and a halogenated ether.

7. A method according to anyone of claims 1 to 6, wherein the lipid soluble process chemical is an inducer of lymphokine synthesis.

8. A method according to anyone of claims 1 to 7, wherein the biological material is selected from the group consisting of whole blood plasma, blood serum, cryodepleted plasma and cryoprecipitate.

9. A method according to anyone of claims 1 to 7, wherein the biological material is a cell.

10. A method according to anyone of claims 1 to 9, wherein the biological material is selected from the group consisting of blood plasma, blood serum, cryoprecipitate, cryodepleted serum, Fraction I, Fraction II, Fraction III, Fraction IV-1, Fraction IV-4, Fraction V, Fraction VI, fibronectin, antihemophilic factor, prealbumin, retinol-binding protein, albumin, alpha-globulins, beta-globulins, gamma globulins, antithrombin III, prothrombin, plasminogen, fibrinogen, factor XIII, immunoglobulin G, immunoglobulin A, immunoglobulin M, immunoglobulin D and immunoglobulin E, plasmin inhibitor, prothrombin, thrombin, antithrombin, factor V, factor VII, factor VIII, factor IX, factor X, normal cells, cancer cells, exudate from cancer cells grown in culture, exudate from normal cells grown in culture, cells from hybridomas, products of gene splicing, plant cell concentrates, plant cell suspensions, extracts of animal tissue, extracts of plant tissue and microorganisms.

11. A method of improving the filterability and/or stability of biological fluids by removing exogenous or endogenous lipid soluble compounds from a biological material containing said lipid soluble compounds, the method comprising passing said biological material containing said lipid soluble compounds through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological

material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.

12. A method according to claim 11, characterized by at least one of the following features:

- (a) the resin and the silica matrix are defined as in claim 2,
- 5 (b) the resin is activated with an organic solvent as defined in claim 3,
- (c) the column is operated at a flowrate as defined in claim 4,
- (d) the method is conducted at a temperature as defined in claim 5.

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European Patent
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EUROPEAN SEARCH REPORT

Application Number

EP 89 11 8199

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	ANALYTICAL BIOCHEMISTRY vol. 109, 1980, pages 207-215; A. FURTH: "Removing unbound Detergent from Hydrophobic Proteins" * Page 210, last paragraph * ---	1,4,6	B 01 D 15/08 A 61 K 35/16
A	PIERCE HANDBOOK & GENERAL CATALOG 1989 * Pages 266-267 * ---	1	
A	EP-A-0 218 374 (INTERN. MINERAL & CHEM. C.) * Column 3, line 32 - column 4, line 37 * ---	1	
A	EP-A-0 263 536 (CETUS CORP.) ---		
A	US-A-4 485 017 (TAN) * Column 3, line 57 - column 4, line 5 * ---	1	
A,P	CHEMICAL ABSTRACT; vol. 111, 1989, abstract no. 19908j, Columbus, Ohio, US; STAFFAN: "Rapid Purification of detergent-solubilized bovine hormone-sensitive lipase by high performance hydrophobic interaction chromatography", & BIOMED: CHROMATORGR. 1989, 3(2), 82-7 * Abstract * -----	1	TECHNICAL FIELDS SEARCHED (Int. Cl.5) B 01 D C 07 K
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 07-02-1990	Examiner WENDLING J.P.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			

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FAN: "Rapid Purification of detergent-
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Description

The present invention concerns the removal of process chemicals from labile biological mixtures by hydrophobic interaction chromatography. More particularly, the present invention concerns the removal of lipid soluble process chemicals, for example, chemicals used in the inactivation of viruses in plasma, cryoprecipitates and plasma-derived therapeutic preparations.

Blood can contain each of several different viruses including but not limited to hepatitis B virus (HBV), non-A, non-B hepatitis virus (NANBHV), cytomegalovirus, and immunodeficiency viruses. It is highly desirable to inactivate these viruses in the course of preparing blood products and prior to the therapeutic application of blood and blood fractions. Both physical (e.g., heat, irradiation) and chemical (e.g., aldehydes, organic solvents, detergents, etc.) methods have been used to inactivate viruses such as HBV in mammalian blood and blood fractions. Inactivation renders a virus non-infectious and non-pathogenic.

Numerous attempts have been made to inactivate viruses such as hepatitis B virus (HBV) in mammalian, especially human, blood plasma. It is the practice in some countries to effect inactivation of the hepatitis B virus in the blood plasma by bringing the plasma into contact with a viral inactivating agent which crosslinks the proteinaceous portions of hepatitis B virus or which interacts with the nucleic acid of the virus. For instance, it is known that hepatitis B virus is inactivated by contact with an aldehyde, such as formaldehyde.

Among the procedures for inactivating viruses are the use of lipid solvents with the addition of surface active agents (A. M. Prince, B. Horowitz, B. Brotman et al, "Inactivation of Hepatitis B and Hutchinson Strain non-A, non-B Hepatitis Viruses by Exposure to Tween 80 and Ether", *Vox Sang*, (1984), 46, 36-43; A. M. Prince, B. Horowitz and B. Brotman, "Sterilisation of Hepatitis and HTLV-III Viruses By Exposure to Tri(n-Butyl)Phosphate and Sodium Cholate", *The Lancet*, 706-710, March 29, 1986), and lipid solvents without the additive of surface active agents (S. M. Feinstone, K. B. Mihalik, T. Kamimura et al, "Inactivation of Hepatitis B Virus and non-A, non-B Hepatitis by Chloroform", *Infect. Immunol.*, (1983), 41, 816-821; D. W. Bradley, J. E. Maynard, H. Popper et al, "Posttransfusion non-A, non-B Hepatitis: Physicochemical Properties of Two Distinct Agents", *J. Infect. Dis.*, (1983), 148, 254-265).

U.S. patents 4,481,189 and 4,540,573 describe the use of organic solvent/detergent pairs to reduce the infectivity of hepatitis viruses and certain other viruses contained in or added to plasma and plasma products by several orders of magnitude. An example of such a pair is tri-n-butyl phosphate, an organic solvent, and TRITON X-100, a non-ionic detergent.

Solvent/detergent treatment under appropriate conditions of temperature and contact time effectively disassembles viruses that have envelope proteins associated with lipid, while having negligible effect on the molecular conformation and biological activities of sensitive blood plasma proteins. The independent effects of organic solvents and detergents in disassembling and attenuating viruses can be facilitated by the presence of both. Merocyanine, beta-propiolactone and cis-platin are among other agents that are applied to blood to inactivate viruses, though by mechanisms other than envelope disruption.

Removal of organic solvents, detergents and other virus-inactivating agents from biological products is necessary if a particular substance is not well tolerated by humans or other biological systems in which it is to be used, e.g., in tissue cultures. In the preparation of purified plasma proteins such as coagulation factor VIII or mixtures of selected proteins, the separation of the desired product from the virus-inactivating agents is often facilitated by a purification process. Thus, precipitation of the desired protein or positive adsorption by an immobilized product-specific ligand can often reduce the level of residual agents to "tolerable" levels by allowing the majority to be washed away.

Other methods used to achieve removal of lipid/detergent micelles from membrane-protein complexes may be applicable to removal of the same from plasma products and other biologic products. These have been based on differences in size, buoyant density, charge, binding affinity, phase partitioning and solvent partitioning (A. Helenius and K. Sinous, "Solubilization of Membranes by Detergents", *Biochem. Biophys. Acta*, (1975), 415, 29-79).

In the instance of whole blood plasma, blood serum, cryodepleted plasma or cryoprecipitate for direct use in transfusion, implementation of the organic solvent/ detergent method of virus sterilization has not occurred. Because preparation of these materials does not involve steps for fractionation, purification, or refinement, there is no convenient opportunity to effect removal of the inactivating agents by the methods suggested above. An efficient removal method is further constrained by the necessity of leaving the composition and biological activity of the preparation substantially intact.

Another difficulty in preparing virus sterilized plasma and cryoprecipitate is in filtering the plasma after treatment to maintain bacterial sterility without loss of labile proteins and biological activity.

Thus, because virus sterilization techniques have not been applied to whole blood plasma, etc., virus infectivity upon infusion remains, estimated at 0.05% for hepatitis B and 3% for non-A, non-B hepatitis transmission.

Exogenous chemicals are frequently added to biological mixtures to stimulate synthesis, inactivate
 5 viruses contained therein and to stabilize or purify desired components present in the mixture. It is desirable to remove these chemicals without otherwise affecting the structure and function of the desired components. For example, the synthesis of certain desired biological products can be induced or enhanced in cell cultures by introduction of phorbol esters into the culture fluid. For example, mezerein may be used to induce gamma interferon production by cultured leukocytes (Y. K. Yip, R. H. L. Pang, J. O. Oppenheim, M.
 10 S. Nashbar, D. Henriksen, T. Zerebeckyj-Eckhardt, J. Vilcek, "Stimulation of Human Gamma Interferon Production by Diterpene Esters", *Infect. and Immun.* (1981) 131-139) or to augment secretion of tumor necrosis factor by cells that produce it (B. D. Williamson, E. A. Carswell, B. Y. Rubin, J. S. Prendergast, H. J. Old, "Human Tumor Necrosis Factor Produced by Human B-cell Lines: Synergistic Cytotoxic Interaction with Human Interferon", *Proc. Natl. Acad. Sci., USA*, (1983), 80, 5397-5401).

15 Before use in man, phorbol esters must be removed from lymphokine preparations because of the carcinogenic properties of these compounds. Heretofore, phorbol esters have been removed by precipitation, chromatographic, or molecular exclusion processes, (B. Y. Rubin, S. L. Anderson, S. A. Sullivan, B. D. Williamson, E. A. Carswell, L. J. Old, "Purification and Characterization of a Human Tumor Necrosis Factor from the LukII Cell Line", *Proc. Natl. Acad. Sci., USA*, (1985), 82, 6637-6641).

20 A method for removal of TNBP and other lipid soluble process chemicals from complex biological mixtures by extraction with vegetable oils, e.g., soy bean oil, is described in European Patent Application 87 103 212, filed March 17, 1987. This method does not remove most detergents.

A similar method for removal of TNBP, detergents and other lipid soluble chemicals by extraction with long-chain alcohols or halogenated esters is described in pending European Patent Application 88 121 582,
 25 filed December 23, 1988. Shortcomings of this method are that expensive and/or noxious chemicals are required and that additional steps must be taken to reduce to tolerable levels any residual extraction agents. An alternative method that efficiently removed TNBP and detergents and did not present these problems would be desirable.

Hydrophobic interaction chromatography (HIC) is a commonly used tool in the biochemists' arsenal of
 30 molecular separation techniques. A description of the principles of HIC is given in S. Hjerten, "Some General Aspects of Hydrophobic Interaction Chromatography", *J. of Chromatography*, (1973), 87, 325-331. Briefly, a lipid-like moiety such as an alkyl chain coupled to an inert matrix is used to partition molecules containing similar hydrophobic domains from aqueous solutions by virtue of their mutual affinity. The alkyl chain may range from two to twenty-four or more carbons in length and may be linear or branched and may
 35 contain or terminate in other hydrophobic groups such as a phenyl ring. Increasing chain length results in media with greater hydrophobic character.

In practice, the strength of the hydrophobic interactions is also influenced by the ionic strength, pH and polarity of the solvent. For example, a high concentration (i.e., 4 molar) of ammonium sulphate in the solvent would promote hydrophobic interaction and, hence, binding between the resin and the hydrophobic
 40 domains of the solute proteins. Following absorption, the proteins can be eluted from the resin by using a buffer with lower ionic strength, chaotropic ions, and/or polarity-lowering additives, such as ethylene glycol or detergents. In addition to resins that react strictly through hydrophobicity, there are materials that work via a combination of hydrophobic and ionic interactions such as amino-hexyl Sepharose (LKB/Pharmacia, Piscataway, N.J.) which incorporates an amino group at the end of a six carbon alkyl chain as the active
 45 function. Among the many plasma proteins that have been purified by these methods are albumin, transferrin, thyroglobulin, lactic dehydrogenase, beta lipoproteins, coagulation factors and immunoglobulins.

The inert matrix to which the hydrophobic groups are bound in the preparation of HIC media may be comprised of a polysaccharide, such as agarose or silica or other polymers. Agarose based media are relatively soft and, in a typical chromatography system, require slow flow rates and result in lengthy
 50 separation procedures. Silica based media, being incompressible, are typically employed in high pressure chromatography systems, but may be used in low pressure systems at relatively high flow rates.

Though generally used for the isolation of proteins, hormones, and other biological molecules from complex biological mixtures, HIC has also been used to remove detergent from certain biological prepara-

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** US patent application 07/139,502

tions where the detergent was used to dissociate membranes. For example, C18 HIC medium in a cartridge (Sep-Pak, Waters Associates, Milford, M.A.), as well as an ion exchange column was used to remove TRITON X-100, protein, salts and other interfering compounds from rat brain homogenates in order to facilitate the measurement of inositol in the tissue extract (S.E. Laursen, H.R. Knull and J.K. Belknap, "Sample Preparation for Inositol Measurement: Sep-Pak C18 Use in Detergent Removal", Analytical Biochemistry, 153, 387-390 (1986)).

It has now been discovered, quite surprisingly, that HIC, normally performed under stringent conditions, such as high salt concentration in the loading solvent for the isolation of proteins, hormones and other substances from biological preparations, can be used under mild conditions - that is, at the native concentrations of proteins, salts and other physiologic constituents in biological fluids - to extract organic solvents and detergents added to these fluids for the purpose of inactivating viral contaminants.

It was further surprising to find that certain constituents of blood plasma, cryoprecipitate, etc., such as coagulation factors and other enzymes - proteins particularly sensitive to denaturation or inactivation or adsorption by hydrophobic media - were present at high concentration and biological activity following removal of virus inactivating agents by the present invention.

Also surprising was the discovery that when long chain alcohols, particularly 2-octanol or a halogenated ether, particularly isoflurane, or a combination of the two were used in purification of biological materials to extract virus inactivating agents, residuals of these compounds could be reduced to trace levels by application of HIC.

It is an object of the present invention to remove virus inactivating solvents and/or detergents and/or other process chemicals, such as phorbol esters, from biological materials, without destroying the properties of the desired components. This object and other objects are provided by the present invention wherein lipid soluble process chemicals, e.g., virus inactivating solvents and/or certain detergents and/or phorbol esters, are removed from biological materials, e.g., labile biological materials, by passing such biological materials containing such lipid soluble process chemicals through a hydrophobic interaction chromatography (HIC) column having as a packing a resin of C-6 to C-24 chains. Preferably the hydrophobic interaction chromatography is employed using octadecyl (C-18) chains coupled to a silica matrix support. The method of the present invention is particularly useful in removing tri-n-butyl phosphate (TNBP) and "TRITON X" detergent from complex biological mixtures such as plasma, cryoprecipitate, or AHF concentrate with little or no loss of coagulation factor activity and minimal change in protein composition.

In the present inventive method, the biological material substantially retains its activity. Furthermore, little or no biological material is absorbed during the passing through the column.

The present invention concerns methods for removing virus attenuating solvents from biological materials to which such solvents have been added. The present invention also concerns removal of certain virus attenuating detergents from biological materials to which such detergents have been added together with or without solvents.

The present invention still further concerns methods for removing other virucidal agents from biological materials.

The present invention further concerns methods for removing process chemicals added as stabilizers to biological materials.

The present invention also further concerns methods for removing process chemicals used in the purification of biological materials.

The present invention additionally concerns methods for removing naturally occurring lipids and other endogenous or exogenous, e.g., "TRITON X-45" and TNBP, lipid soluble compounds from biological materials, such removal resulting in improved properties of said materials, e.g., filterability, stability, or rendering virus sterilization reagents more effective.

Accordingly, the present invention concerns a method of removing lipid soluble process chemicals from a biological material and a method of improving the filterability and/or stability of biological fluids by removing exogenous or endogenous lipid soluble compounds from a biological material containing lipid soluble compounds, the method comprising passing a biological material containing lipid soluble compounds through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.

The present invention also concerns methods for the removal of chemical inducers such as lymphokine inducing phorbol esters (i.e., inducers of lymphokine synthesis) from biological materials.

The present invention further concerns the removal of process agents added to blood cells, without disruption of the cells.

Blood is made up of solids (cells, i.e., erythrocytes, leucocytes, and thrombocytes) and liquid (plasma). The cells contain potentially valuable substances such as hemoglobin, and they can be induced to make other potentially valuable substances such as interferons, growth factors, and other biological response modifiers. The plasma is composed mainly of water, salts, lipids and proteins. The proteins are divided into groups called fibrinogen, serum globulins and serum albumin. Typical antibodies (immune globulins) found in human blood plasma include those directed against infectious hepatitis, influenza H, etc.

Cells found in blood include red cells, various types of leukocytes or white cells, and platelets. Fractionation of cell types typically utilizes centrifugation, but may involve other forms of differential sedimentation through addition of rouleaux enhancing agents such as hydroxyethyl starch, separations based on immunological specificity, etc.

Proteins found in human plasma include prealbumin, retinol-binding protein, albumin, alpha-globulins, beta-globulins, gamma-globulins (immune serum globulins), the coagulation proteins (antithrombin III, prothrombin, plasminogen, antihemophilic factor (factor VIII), fibrin-stabilizing factor (factor XIII), fibrinogen, immunoglobins (immunoglobulins G, A, M, D, and E), and the complement components. There are currently more than 100 plasma proteins that have been described. A comprehensive listing can be found in "The Plasma Proteins", ed. Putnam, F.W., Academic Press, New York (1975).

Proteins found in the blood cell fraction include hemoglobin, fibronectin, fibrinogen, enzymes of carbohydrate and protein metabolism, platelet derived growth factor etc. In addition, the synthesis of other proteins can be induced, such as interferons and growth factors.

A comprehensive list of inducible leukocyte proteins can be found in Stanley Cohen, Edgar Pick, J.J. Oppenheim, "Biology of the Lymphokines", Academic Press, N.Y. (1979).

Blood plasma fractionation generally involves the use of organic solvents such as ethanol, ether and polyethylene glycol at low temperatures and at controlled pH values to effect precipitation of a particular fraction containing one or more plasma proteins. The resultant supernatant can itself then be precipitated and so on until the desired degree of fractionation is attained. More recently, separations are based on chromatographic processes. An excellent survey of blood fractionation appears in Kirk-Othmer's Encyclopedia of Chemical Technology, Third Edition, Interscience Publishers, Volume 4, pages 25 to 62.

The major components of a cold ethanol fractionation are as follows:

Fraction	Proteins
I	fibrinogen; cold insoluble globulin; factor VIII; properdin
II and III	IgG; IgM; IgA; fibrinogen; beta-lipoprotein; prothrombin; plasminogen; plasmin inhibitor; factor V; factor VII; factor IX; factor X; thrombin; antithrombin; isoagglutinins; ceruloplasmin; complement C'1, C'3
IV-1	alpha ₁ -lipoprotein, ceruloplasmin; plasmin-inhibitor; factor IX; peptidase; alpha-and-beta-globulins
IV-4	transferrin; thyroxine binding globulin; serum esterase; alpha ₁ -lipoprotein, albumin; alkaline phosphatase
V	albumin; alpha-globulin
VI	alpha ₁ -acid glycoprotein; albumin

The above fractionation scheme can serve as a basis for further fractionations. Fraction II and III, for example, can be further fractionated to obtain immune serum globulin (ISG).

Another fractionation scheme involves use of frozen plasma which is thawed into a cryoprecipitate containing AHF (antihemophilic factor) and fibronectin and a cryosupernatant. The cryoprecipitate is then fractionated into fibronectin and AHF.

The methods of the present invention are applicable to biological materials including blood cells, blood plasma, blood fractions thereof, and blood proteins such as those discussed hereinabove, cryoprecipitate, cryodepleted serum and more generally to biological cells and fluids, e.g., normal cells, cancer cells, exudate from cancer cells grown in culture, exudate from normal cells grown in culture, cells from hybridomas, products of gene splicing, plant cell concentrates, plant cell suspensions, extracts of animal tissue, extracts of plant tissue and microorganisms.

Examples of organic long chain alcohols for use in the present invention include hexanol, heptanol, 1-octanol, 2-octanol, 1-nonanol, 1-decanol and undecanol.

Examples of halogenated (e.g., containing fluorine, chlorine, iodine and/or bromine) hydrocarbons for use in the present invention include 1,2,2-trifluorotrichlorethane, "ETHRANE" (enflurane; 2-chloro-1,1,2-trifluoroethyl difluoromethyl ether), "FORANE" (isoflurane; 1-chloro-2,2,2-trifluoroethyl difluoromethyl

ether). Preferred halogenated hydrocarbons according to the present invention contain fluorine, chlorine and ether.

The typical solvents removed by the present invention are liquid at the temperature for use, immiscible with the aqueous solutions being extracted, non-denaturing to proteins and to cells under the conditions of use, easily removed, non-explosive, and non-toxic in the quantities remaining in the biological solution under the conditions of use.

Di- or trialkylphosphates, detergents and surfactants for removal by the process of the present invention are described in U.S. Patents 4,540,573 and 4,481,189.

Ranges for solvent and detergent encountered in treated biological materials to be subjected to HIC according to the present invention are as follows:

solvent	1000-20,000 ppm
detergent	1000-10,000 ppm.

The present invention is particularly directed, *inter alia*, to producing a protein-containing composition such as blood plasma, cryoprecipitates, blood plasma fractions, etc., which is substantially free of infectious virus, yet which retains a substantial amount of enzymatically or biologically active (undenatured) protein and from which process chemicals have been removed so that the resultant composition has no more than physiologically acceptable levels of such process chemicals.

Biological fluids for use according to the present invention include blood plasma, blood plasma fractions, precipitates from blood fractionation and supernatants from blood fractionation. Also contemplated is the treatment of concentrates of whole blood cells, red cells, white cells (leukocytes), platelets, platelet rich plasma, platelet poor plasma, and concentrates of granulocytes, monocytes, or lymphocytes or other cells capable of producing interferon, tumor necrosis factor (TNF), or other immune modulators or lymphokines, or the media separated from such concentrates or suspension.

According to the present invention, there is contemplated the preparation of a protein-containing composition, particularly whole blood plasma or whole blood serum having an extent of inactivation of virus greater than 6 logs of virus, such as AIDS virus (HIV I), hepatitis B virus and non-A non-B hepatitis virus, having a retention of functional activity for particularly biologically active proteins of at least 45%, preferably at least 75%, more preferably at least 85%, even more preferably at least 95% and most preferably 98% to 100%, and having no more than physiologically acceptable levels of lipid soluble process chemicals.

Coagulation factor activity is retained at more than 60% of its original level and preferably more than 75 to 85%, and most preferably at more than 90 to 98% of its original level.

The (virus sterilized) whole blood plasma, blood serum, cryoprecipitate or cryodepleted plasma according to the present invention can be transfused directly into a patient, e.g., mammal, e.g., human. Alternatively, the (virus sterilized) whole blood plasma, blood serum, cryodepleted plasma or cryoprecipitate according to the present invention can be fractionated to prepare purified plasma protein derivatives (such derivatives can be transfused directly into a patient, e.g., a human patient).

The whole blood plasma or blood serum according to the present invention can also be used in cell cultures and as a quality control reagent.

Furthermore, non-blood sources including, for example, normal (noncancerous) or cancer cells, exudate from cancer or normal cells grown in culture, hybridomas and products from gene splicing, plant cell concentrates or suspensions, extracts of animal or plant tissues, or microorganisms can be used as the biological fluid in the present invention.

The process of the present invention differs from other applications of HIC in that materials and conditions are employed that minimize adsorption and separation of proteins and maximize the removal of lipid-soluble process chemicals as described.

The preferred resin for use in the present invention is Bulk C-18 packing from Waters, Inc. having a particle size of 55-105 microns and a porosity of 120 Angstroms. The active function is an eighteen carbon linear chain coupled to a silica matrix. The uncoupled sites on the matrix are blocked with dimethylsilane.

The capacity of this material for binding TRITON X-100 detergent is approximately 160 mg (0.25 millimoles) of detergent per gram of dry resin. Other C-18 resins from Waters such as Bondapack C-18 or Megabond C-18 of their equivalents from other manufacturers provide similar capacity. The use of a silica matrix permits the extraction process to occur at higher flow rates and at higher pressures than are obtainable with more compressible chromatography media. Typically, columns can be operated at a flow rate of 125 to 175 ml/cm²/hr or higher compared to 25 to 50 ml/cm²/hr for an agarose-based resin.

In practice, resin is preferably packed into a stainless steel or glass chromatography column. The column volume should preferably be at least 1/8 of the volume of the material to be loaded if the "TRITON" concentration in the material is 1% (w/v). The resin is activated by washing with isopropanol and then with water. Ethanol and acetonitrile are suitable organic phases for activation and regeneration of the resins.

5 Before loading the column is preferably equilibrated with saline or an appropriate buffer.

The temperature range for the process is preferably from 4° to 37° C and most preferably 20° C to 25° C.

No more than 15 weight % of the biological material is adsorbed on the column and preferably no more than 5 to 10% is adsorbed. Most preferably, no more than 2 to 5% is adsorbed on the column.

10 The column is preferably cleaned and regenerated after absorbing the virus-inactivating agents by washing with water and increasing concentrations of ethanol or isopropanol from 15% to 100% and then water again. If ethanol is used, a column volume of 100% isopropanol should also preferably be used afterward.

Material to be processed according to the invention includes, for example, plasma, cryoprecipitate, AHF
 15 concentrate, immune globulin and Prothrombin Complex containing TNBP and "TRITON X-100". Other complex protein mixtures such as vaccines, coagulation factors, serum, etc. can be processed as described herein.

The present invention will now be described with reference to the following examples.

20 EXAMPLES

Example 1: Preparation of Virus-Sterilized Plasma

Six units of fresh frozen plasma were thawed and pooled. TNBP and TRITON X-100 were added to a
 25 concentration of 1% (w/v) and the solution was incubated for 4 hours at 37° C with gentle agitation. Following the incubation, the material was clarified, if necessary, by centrifugation at 10,000 x g. The plasma was then passed through a column containing 60 g of Bulk C-18 media (Waters, Inc., Milford, MA.) which had previously been washed with several column volumes of isopropanol followed by sterile saline. The flow rate through the column was approximately 150 ml/cm² per hour and the operating temperature
 30 was 23-25° C. After processing the plasma, the column was regenerated by washing with saline followed by a gradient of 15 to 95% ethanol, followed in turn by 100% isopropanol. Table 1, below, illustrates the effectiveness of TNBP and TRITON removal and the recovery of coagulation factors V and VIII in eight batches of plasma processed as described. The activated partial thromboplastin time (APTT) is a comprehensive measure of clotting factor activity. The results in Table 1 indicate that 80 to 95% of clotting
 35 factor activity is retained. Table 2 shows the minor differences that exist in a pool of plasma before and after treatment as described with regard to a number of protein fractions, enzymes, and other constituents. In general, the levels of all of the constituents measured are within the normal physiological range. However, the lipid content is lower, resulting in increased filterability.

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Table 1. Summary of Virus Sterilized Plasma Preparations

5	BATCH	1	2	3	4	5	6	7	8	AVG.
	TNBP (ppm)	5.0	1.6	7.7	1.4	2.1	0.8	1.5	2.7	2.85
	TRITON (ppm)	4.0	8.0	8.0	1.0	5.0	8.0	2.0	1.5	4.69
10	FACTOR VIII (units/ml)									
	starting pool	0.96	1.08	1.13	1.23	1.13	0.99	1.12	1.34	1.12
	after treatment	1.01	1.00	0.75	0.95	0.85	0.89	0.96	0.83	0.91
15	FACTOR V (units/ml)									
	starting pool	1.05	0.88	1.08	1.04	1.18	1.11	1.04	0.78	1.02
20	after treatment	0.96	0.89	0.66	0.81	1.03	1.01	0.85	0.70	0.86
	APTT (seconds)									
	starting pool	31.2	30.5	29.3	29.2	29.5	29.5	28.5	29.2	29.6
25	after treatment	31.8	31.5	nd	30.8	38.3	nd	29.2	32.1	32.3
	nd = no data									

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Table 2

Some Characteristics of Plasma Before and After Virus-Sterilization			
		UNTREATED	TREATED
TOTAL PROTEIN	g/dl	7.3	6.5
ALBUMIN	g/dl	4.2	4.2
IgG	mg/dl	1310	1200
IgA	mg/dl	249	192
IgM	mg/dl	183	90
C3	mg/dl	79	62
C4	mg/dl	33.4	22.1
HAPTOGLOBIN	mg/dl	130	109
TRIGLYCERIDES	mg/dl	176	104
CHOLESTEROL	mg/dl	195	93
CALCIUM	mg/dl	8	8.1
PHOSPHORUS	mg/dl	12	12
IRON	μg/dl	182	199
BUN	mg/dl	17	15
URIC ACID	mg/dl	4.9	4.5
TOTAL BILIRUBIN	mg/dl	0.3	0.3
CREATININE	mg/dl	1.2	0.8
LDH	units/ml	173	181
ALT	units/ml	23	22
GGT	units/ml	19	19
AMYLASE	units/ml	32	30
ALK PHOS	units/ml	100	52
SGOT	units/ml	39	40
BUN = blood urea nitrogen ALT = alanine amino transferase GGT = gamma-glutamyl transpeptidase ALK PHOS = alkaline phosphatase SGOT = serum glutamic oxaloacetic transaminase LDH = lactic dehydrogenase			

Example 2: Removal of TNBP and "TRITON" from preparations of several products:

Each product was inactivated by incubation with TNBP (1% for plasma and cryo, 0.3% for other products) and 1% "TRITON X-100" for at least 3 hours. Material was clarified, if necessary, by centrifugation at 10,000 x g before loading on column. The column contained 12 g of Bulk C-18 packing (Waters, Inc.) which had been activated with several column volumes of organic phase (either isopropanol, acetonitrile, or ethanol) and then washed with several volumes of distilled water prior to loading of sample. In Table 3 below, recovery refers to the yield on the column step.

Table 3

Material Processed	Triton (ppm)	TNBP (ppm)	Recovery (%)	measured by
plasma-forane extr.	5.4	1.0	90	APPT
AHF concentrate	2.5	3.3	83	AHF activity
ISG	12.2	2.3	99	Total protein
Cryoprecipitate	7.0	0.6	86	AHF activity
extr. = plasma was extracted with forane prior to loading on the C ₁₈ column				
ISG = immune serum globulin				

Claims

1. A method of removing lipid soluble process chemicals from a biological material containing said lipid soluble chemicals, said method comprising passing said biological material containing said lipid soluble chemicals through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.
2. The method according to claim 1 wherein, prior to passing said biological material through said column, said biological material is separated by centrifugation into a lipid soluble phase and an aqueous phase and said aqueous phase is then passed through said column.
3. The method according to claim 2 wherein a lipid phase extracting chemical is added prior to separating said lipid soluble phase from said aqueous phase.
4. The method according to claim 1 wherein a lipid phase extracting chemical is added prior to passing said biological material through said column.
5. The method according to any one of claims 1 to 4 wherein the resin comprises an active function and a support and wherein the active function comprises octadecyl chains and the support comprises a silica matrix wherein the silica matrix preferably has uncoupled sites which are blocked with dimethylsilane.
6. The method according to any one of claims 1 to 5 wherein the resin is activated with an organic solvent selected from isopropanol, ethanol or acetonitrile.
7. The method according to any one of claims 1 to 6 wherein the column is operated at a flowrate of 125 to 175 ml/cm²/hour.
8. The method according to any one of claims 1 to 7 wherein the method is conducted at a temperature of 4° to 37°C, preferably from 20° to 25°C.
9. The method according to any one of claims 1 to 8 wherein the lipid soluble process chemicals are selected from an organic solvent, a detergent, a long chain alcohol or a halogenated ether.
10. The method according to any one of claims 1 to 9 wherein the lipid soluble process chemical is TNBP or Triton X 100.
11. The method according to any one of claims 1 to 10 wherein the lipid soluble process chemical is an inducer of lymphokine synthesis.
12. The method according to any one of claims 1 to 11 wherein the biological material is selected from blood plasma, blood serum, cryoprecipitate, cryodepleted serum, Fraction I, Fraction II, Fraction III, Fraction IV-1, Fraction IV-4, Fraction V, Fraction VI, fibronectin, antihemophilic factor, prealbumin, retinol-binding protein, albumin, alpha-globulins, beta-globulins, gamma globulins, antithrombin III,

- prothrombin, plasminogen, fibrinogen, factor XIII, immunoglobulin G, immunoglobulin A, immunoglobulin M, immunoglobulin D, immunoglobulin E, plasmin inhibitor, thrombin, antithrombin, factor V, factor VII, factor VIII, factor IX, factor X, normal cells, cancer cells, exudate from cancer cells grown in culture, exudate from normal cells grown in culture, cells from hybridomas, products of gene splicing, plant cell concentrates, plant cell suspensions, extracts of animal tissue, extracts of plant tissue or microorganisms.
13. The method according to any one of claims 3 to 12 wherein the lipid phase extracting chemical is a long chain alcohol or a halogenated ether.
14. The method according to claim 13 wherein the lipid phase extracting chemical is 2-octanol or isofluorane.
15. A method of improving the filterability and/or stability of biological fluids by removing exogenous or endogenous lipid soluble compounds from a biological material containing said lipid soluble compounds, said method comprising passing said biological material containing said lipid soluble compounds through a hydrophobic interaction chromatography column containing C-6 to C-24 resin, wherein the biological material has biological activity and wherein the biological activity is substantially retained, and wherein little or no biological material is adsorbed during said passing through said column.
16. The method according to claim 15, characterized by at least one of the following features:
- (a) the resin and the silica matrix as defined in claim 5,
 - (b) the resin is activated with an organic solvent as defined in claim 6,
 - (c) the column is operated at a flowrate as defined in claim 7, and
 - (d) the method is conducted at a temperature as defined in claim 8.
17. A method for the production of blood plasma which is substantially free of infectious viruses comprising treating the plasma with virus-inactivating solvents and/or detergents and removing the virus-inactivating solvents and/or detergents by hydrophobic interaction chromatography on a C-6 to C-24 resin.
18. The method according to claim 17 wherein, prior to removing said virus-inactivating solvents and/or detergents by hydrophobic interaction chromatography, said plasma is separated by centrifugation into a lipid soluble phase and an aqueous phase and said virus-inactivating solvents and/or detergents are then removed from said aqueous phase by said hydrophobic interaction chromatography.
19. The method according to claim 18 wherein a lipid phase extracting chemical is added prior to separating said lipid soluble phase from said aqueous phase.
20. The method according to claim 17 wherein a lipid phase extracting chemical is added prior to removing said virus-inactivating solvents and/or detergents by said hydrophobic interaction chromatography.
21. The method according to any one of claims 17 to 20 wherein the resin comprises an active function and a support and wherein the active function comprises octadecyl chains and the support comprises a silica matrix wherein the silica matrix preferably has uncoupled sites which are blocked with dimethylsilane.
22. The method according to any one of claims 17 to 21 wherein the resin is activated with an organic solvent selected from isopropanol, ethanol or acetonitrile.
23. The method according to any one of claims 17 to 22 wherein the column is operated at a flowrate of 125 to 175 ml/cm²/hour.
24. The method according to any one of claims 17 to 23 wherein the method is conducted at a temperature of 4° to 37° C, preferably from 20° to 25° C.
25. The method according to any one of claims 17 to 24 wherein the lipid soluble process chemicals are selected from an organic solvent, a detergent, a long chain alcohol or a halogenated ether.

26. The method according to any one of claims 17 to 25 wherein the lipid soluble process chemical is TNBP or Triton X 100.
27. The method according to any one of claims 17 to 26 wherein the lipid soluble process chemical is an inducer of lymphokine synthesis.
28. The method according to any one of claims 19 to 27 wherein the lipid phase extracting chemical is a long-chain alcohol or a halogenated ether.
29. The method according to claim 28 wherein the lipid phase extracting chemical is 2-octanol or isofluorane.

Patentansprüche

1. Verfahren zum Entfernen von lipidlöslichen Verfahrenskemikalien aus einem diese lipidlöslichen Chemikalien enthaltendem biologischen Material, wobei das diese lipidlöslichen Chemikalien enthaltende biologische Material durch eine C-6 bis C-24 Harz enthaltende hydrophobe Austauschchromatographie-Säule laufen gelassen wird, das biologische Material biologische Aktivität aufweist, die biologische Aktivität im wesentlichen erhalten bleibt und wenig oder kein biologisches Material während des Durchlaufs durch die Säule adsorbiert wird.
2. Verfahren nach Anspruch 1, wobei vor Durchlauf des biologischen Materials durch die Säule das biologische Material durch Zentrifugation in eine lipidlösliche und eine wäßrige Phase getrennt wird und anschließend die wäßrige Phase durch die Säule laufen gelassen wird.
3. Verfahren nach Anspruch 2, wobei eine Lipidphasen-extrahierende Chemikalie zugegeben wird, bevor die lipidlösliche von der wäßrigen Phase getrennt wird.
4. Verfahren nach Anspruch 1, wobei eine Lipidphasen-extrahierende Chemikalie zugegeben wird, bevor das biologische Material über die Säule gegeben wird.
5. Verfahren nach einem der Ansprüche 1 bis 4, wobei das Harz eine aktive Funktion und einen Träger umfaßt, und wobei die aktive Funktion Octadecylketten und der Träger eine Silika-Matrix umfaßt, wobei die Silika-Matrix vorzugsweise ungebundene Stellen aufweist, die mit Dimethylsilan blockiert sind.
6. Verfahren nach einem der Ansprüche 1 bis 5, wobei das Harz mit einem organischen Lösungsmittel ausgewählt aus Isopropanol, Ethanol oder Acetonitril aktiviert wird.
7. Verfahren nach einem der Ansprüche 1 bis 6, wobei die Säule eine Fließrate von 125 bis 175 ml/cm²/Std. aufweist.
8. Verfahren nach einem der Ansprüche 1 bis 7, wobei das Verfahren bei einer Temperatur von 4° bis 37° C, vorzugsweise von 20° bis 25° C durchgeführt wird.
9. Verfahren nach einem der Ansprüche 1 bis 8, wobei die lipidlöslichen Verfahrenskemikalien ausgewählt sind aus einem organischen Lösungsmittel, einem Detergens, einem langkettigen Alkohol oder einem halogenierten Ether.
10. Verfahren nach einem der Ansprüche 1 bis 9, wobei die lipidlösliche Verfahrenskemikalie TNBP oder Triton X-100 ist.
11. Verfahren nach einem der Ansprüche 1 bis 10, wobei die lipidlösliche Verfahrenskemikalie ein Induktor der Lymphokinsynthese ist.
12. Verfahren nach einem der Ansprüche 1 bis 11, wobei das biologische Material ausgewählt ist aus Blutplasma, Blutserum, Cryopräzipitat, gefriergetrocknetem Serum, Fraktion I, Fraktion II, Fraktion III, Fraktion IV-1, Fraktion IV-4, Fraktion V, Fraktion VI, Fibronectin, antihämophiler Faktor, Präalbumin, Retinol-bindendem Protein, Albumin, α -Globuline, β -Globuline, γ -Globuline, Antithrombin III, Prothrom-

- bin, Plasminogen, Fibrinogen, Faktor XIII, Immunglobulin G, Immunglobulin A, Immunglobulin M, Immunglobulin D, Immunglobulin E, Plasmininhibitor, Thrombin, Antithrombin, Faktor V, Faktor VII, Faktor VIII, Faktor IX, Faktor X, normalen Zellen, Krebszellen, Exsudat aus in Kultur gezüchteten Krebszellen, Exsudat aus in Kultur gezüchteten normalen Zellen, Zellen aus Hybridomen, Produkte aus Gen-Spleißvorgängen, Pflanzenzellkonzentraten, Pflanzenzellsuspensionen, Extrakten aus Tiergewebe, Extrakten aus Pflanzengewebe oder Mikroorganismen.
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13. Verfahren nach einem der Ansprüche 3 bis 12, wobei die Lipidphasen-extrahierende Chemikalie ein langkettiger Alkohol oder ein halogenierter Ether ist.
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14. Verfahren nach Anspruch 13, wobei die Lipidphasen-extrahierende Chemikalie 2-Octanol oder Isofluran ist.
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15. Verfahren zur Verbesserung der Filtrierbarkeit und/oder Stabilität biologischer Flüssigkeiten, wobei exogene oder endogene lipidlösliche Verbindungen aus einem diese lipidlöslichen Verbindungen enthaltenden biologischen Material entfernt werden, und wobei das diese lipidlöslichen Verbindungen enthaltende biologische Material durch eine C-6 bis C-24 Harz enthaltende hydrophobe Austauschchromatographie-Säule laufen gelassen wird, das biologische Material biologische Aktivität aufweist, die biologische Aktivität im wesentlichen erhalten bleibt und wenig oder kein biologisches Material während des Durchlaufs durch die Säule adsorbiert wird.
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16. Verfahren nach Anspruch 15, das durch mindestens eines der folgenden Merkmale gekennzeichnet ist:
- a) das Harz und die Silika-Matrix sind wie in Anspruch 5 angegeben,
 - b) das Harz ist mit einem organischen Lösungsmittel, wie in Anspruch 6 angegeben, aktiviert,
 - 25 c) die Säule weist eine Fließrate wie in Anspruch 7 angegeben auf, und
 - d) das Verfahren wird bei einer Temperatur, wie in Anspruch 8 angegeben, durchgeführt.
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17. Verfahren zur Herstellung von Blutplasma, das im wesentlichen frei von infektiösen Viren ist, umfassend das Behandeln des Plasmas mit Virus-inaktivierenden Lösungsmitteln und/oder Detergentien und Entfernen der Virus-inaktivierenden Lösungsmittel und/oder Detergentien durch hydrophobe Austauschchromatographie auf einem C-6 bis C-24 Harz.
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18. Verfahren nach Anspruch 17, wobei vor Entfernen der Virus-inaktivierenden Lösungsmittel und/oder Detergentien durch hydrophobe Austauschchromatographie das Plasma durch Zentrifugation in eine lipidlösliche und eine wäßrige Phase getrennt wird und die Virus-inaktivierenden Lösungsmittel und/oder Detergentien anschließend aus der wäßrigen Phase durch hydrophobe Austauschchromatographie entfernt werden.
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19. Verfahren nach Anspruch 18, wobei eine Lipidphasen-extrahierende Chemikalie zugesetzt wird, bevor die lipidlösliche von der wäßrigen Phase getrennt wird.
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20. Verfahren nach Anspruch 17, wobei eine Lipidphasen-extrahierende Chemikalie zugesetzt wird, bevor die Virus-inaktivierenden Lösungsmittel und/oder Detergentien durch die hydrophobe Austauschchromatographie entfernt werden.
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21. Verfahren nach einem der Ansprüche 17 bis 20, wobei das Harz eine aktive Funktion und einen Träger umfaßt und wobei die aktive Funktion Octadecylketten und der Träger eine Silika-Matrix umfaßt, wobei die Silika-Matrix vorzugsweise ungebundene Stellen aufweist, die mit Dimethylsilan blockiert sind.
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22. Verfahren nach einem der Ansprüche 17 bis 21, wobei das Harz mit einem organischen Lösungsmittel ausgewählt aus Isopropanol, Ethanol oder Acetonitril aktiviert wird.
23. Verfahren nach einem der Ansprüche 17 bis 22, wobei die Säule eine Fließrate von 125 bis 175 ml/cm²/Std. aufweist.
24. Verfahren nach einem der Ansprüche 17 bis 23, wobei das Verfahren bei einer Temperatur von 4° bis 37° C, vorzugsweise von 20° bis 25° C durchgeführt wird.

25. Verfahren nach einem der Ansprüche 17 bis 24, wobei die lipidlöslichen Verfahrenskemikalien ausgewählt sind aus einem organischen Lösungsmittel, einem Detergens, einem langkettigen Alkohol oder einem halogenierten Ether.
- 5 26. Verfahren nach einem der Ansprüche 17 bis 25, wobei die lipidlösliche Verfahrenskemikalie TNBP oder Triton X-100 ist.
27. Verfahren nach einem der Ansprüche 17 bis 26, wobei die lipidlösliche Verfahrenskemikalie ein Induktor der Lymphokinsynthese ist.
- 10 28. Verfahren nach einem der Ansprüche 19 bis 27, wobei die Lipidphasen-extrahierende Chemikalie ein langkettiger Alkohol oder ein halogener Ether ist.
29. Verfahren nach Anspruch 28, wobei die Lipidphasen-extrahierende Chemikalie 2-Octanol oder Isofluor-
15 ran ist.

Revendications

1. Procédé d'élimination de produits chimiques de traitement solubles lipidiques à partir d'une matière
20 biologique contenant lesdits produits chimiques solubles lipidiques, ce procédé comprenant un passage de la matière biologique contenant les produits chimiques solubles lipidiques à travers une colonne de chromatographie par interaction hydrophobe contenant une résine en C-6 à C-24, procédé dans lequel la matière biologique présente une activité biologique et l'activité biologique est sensiblement maintenue, et peu de matière biologique ou aucune est adsorbée pendant ledit passage à travers la
25 colonne.
2. Procédé suivant la revendication 1, caractérisé en ce que, avant le passage de la matière biologique à travers la colonne, la matière biologique est séparée par centrifugation en une phase soluble lipidique et en une phase aqueuse et en ce que la phase aqueuse est alors passée à travers la colonne.
- 30 3. Procédé suivant la revendication 2, caractérisée en ce qu'un produit chimique d'extraction de phase lipidique est ajouté avant de séparer la phase soluble lipidique de la phase aqueuse.
4. Procédé suivant la revendication 1, caractérisé en ce qu'un produit chimique d'extraction de phase
35 lipidique est ajouté avant de passer la matière biologique à travers la colonne.
5. Procédé suivant l'une quelconque des revendications 1 à 4, caractérisé en ce que la résine comprend une fonction active et un support et en ce que la fonction active comprend des chaînes octadécyle et le support une matrice de silice, la matrice de silice ayant de préférence des sites non couplés qui sont
40 bloqués par du diméthylsilane.
6. Procédé suivant l'une quelconque des revendications 1 à 5, caractérisé en ce que la résine est activée par un solvant organique choisi parmi de l'isopropanol, de l'éthanol ou de l'acétonitrile.
- 45 7. Procédé suivant l'une quelconque des revendications 1 à 6, caractérisé en ce que la colonne est mise en service à un débit de 125 à 175 ml/cm²/heure.
8. Procédé suivant l'une quelconque des revendications 1 à 7, caractérisé en ce que le procédé est réalisé à une température de 4° à 37°C, de préférence de 20° à 25°C.
- 50 9. Procédé suivant l'une quelconque des revendications 1 à 8, caractérisé en ce que les produits chimiques de traitement solubles, lipidiques sont choisis parmi un solvant organique, un détergent, un alcool à longue chaîne ou un éther halogéné.
- 55 10. Procédé suivant l'une quelconque des revendications 1 à 9, caractérisé en ce que le produit chimique de traitement soluble, lipidique est du TNBP ou du Triton X 100.

11. Procédé suivant l'une quelconque des revendications 1 à 10, caractérisé en ce que le produit chimique de traitement soluble, lipidique est un inducteur de synthèse de lymphokine.
- 5 12. Procédé suivant l'une quelconque des revendications 1 à 11, caractérisé en ce que la matière biologique est choisie parmi du plasma sanguin, du sérum sanguin, un cryoprécipité, du sérum cryoappauvri, de la fraction I, de la fraction II, de la fraction III, de la fraction IV-1, de la fraction IV-4, de la fraction V, de la fraction VI, de la fibronectine, un facteur antihémophile, de la préalbumine, de la protéine de fixation de rétinol, de l'albumine, des alpha-globulines, des bêta-globulines, des gamma-globulines, de l'antithrombine III, de la prothrombine, du plasminogène, du fibrinogène, du facteur XIII, 10 de l'immunoglobuline G, de l'immunoglobuline A, de l'immunoglobuline M, de l'immunoglobuline D et de l'immunoglobuline E, un inhibiteur de plasmine, de la thrombine, de l'antithrombine, du facteur V, du facteur VII, du facteur VIII, du facteur IX, du facteur X, des cellules normales, des cellules cancéreuses, un exsudat de cellules cancéreuses multipliées en culture, un exsudat de cellules normales multipliées en culture, des cellules d'hybridomes, des produits d'épissage de gènes, des concentrés de cellules végétales, des suspensions de cellules végétales, des extraits de tissu animal, des extraits de tissu 15 végétal ou des micro-organismes.
13. Procédé suivant l'une quelconque des revendications 3 à 12, caractérisé en ce que le produit chimique d'extraction de phase lipidique est un alcool à longue chaîne ou un éther halogéné.
- 20 14. Procédé suivant la revendication 13, caractérisé en ce que le produit chimique d'extraction de phase lipidique est du 2-octanol ou de l'isofluorane.
- 15 15. Procédé d'amélioration de la filtrabilité et/ou de la stabilité de fluides biologiques par élimination de composés solubles lipidiques exogènes ou endogènes à partir d'une matière biologique contenant ces composés solubles lipidiques, ce procédé comprenant un passage de la matière biologique contenant les composés solubles lipidiques à travers une colonne de chromatographie par interaction hydrophobe contenant une résine en C-6 à C-24, procédé dans lequel la matière biologique présente une activité biologique et l'activité biologique est sensiblement maintenue, et peu de matière biologique ou aucune 30 est adsorbée pendant le passage à travers la colonne.
16. Procédé suivant la revendication 15, caractérisé par au moins une des particularités suivantes :
 - (a) la résine et la matrice de silice sont telles que définies dans la revendication 5,
 - (b) la résine est activée par un solvant organique tel que défini dans la revendication 6,
 - 35 (c) la colonne est mise en service à un débit tel que défini dans la revendication 7, et
 - (d) le procédé est réalisé à une température telle que définie dans la revendication 8.
17. Procédé de production de plasma sanguin qui est sensiblement exempt de virus infectieux, comprenant un traitement du plasma par des solvants et/ou détergents inactivant les virus et une élimination des 40 solvants et/ou détergents inactivant les virus par une chromatographie par interaction hydrophobe sur une résine en C-6 à C-24.
18. Procédé suivant la revendication 17, caractérisé en ce que, avant l'élimination des solvants et/ou détergents inactivant les virus par une chromatographie par interaction hydrophobe, le plasma est 45 séparé par centrifugation en une phase soluble lipidique et en une phase aqueuse et en ce que les solvants et/ou détergents inactivant les virus sont ensuite éliminés de la phase aqueuse par la chromatographie par interaction hydrophobe.
19. Procédé suivant la revendication 18, caractérisé en ce qu'un produit chimique d'extraction de phase 50 lipidique est ajouté avant de séparer la phase soluble lipidique de la phase aqueuse.
20. Procédé suivant la revendication 17, caractérisé en ce qu'un produit chimique d'extraction de phase lipidique est ajouté avant d'éliminer les solvants et/ou détergents inactivant les virus par la chromatographie par interaction hydrophobe.
- 55 21. Procédé suivant l'une quelconque des revendications 17 à 20, caractérisé en ce que la résine comprend une fonction active et un support et en ce que la fonction active comprend des chaînes octadécyle et le support une matrice de silice, la matrice de silice présentant de préférence des sites

non couplés qui sont bloqués par du diméthylsilane.

22. Procédé suivant l'une quelconque des revendications 17 à 21, caractérisé en ce que la résine est activée par un solvant organique choisi parmi de l'isopropanol, de l'éthanol ou de l'acétonitrile.
- 5 23. Procédé suivant l'une quelconque des revendications 17 à 22, caractérisé en ce que la colonne est mise en service à un débit de 125 à 175 ml/cm²/heure.
- 10 24. Procédé suivant l'une quelconque des revendications 17 à 23, caractérisé en ce que le procédé est réalisé à une température de 4° à 37°C, de préférence de 20° à 25°C.
25. Procédé suivant l'une quelconque des revendications 17 à 24, caractérisé en ce que les produits chimiques de traitement solubles, lipidiques sont choisis parmi un solvant organique, un détergent, un alcool à longue chaîne ou un éther halogéné.
- 15 26. Procédé suivant l'une quelconque des revendications 17 à 25, caractérisé en ce que le produit chimique de traitement soluble, lipidique est du TNBP ou du Triton X 100.
- 20 27. Procédé suivant l'une quelconque des revendications 17 à 26, caractérisé en ce que le produit chimique de traitement soluble, lipidique est un inducteur de synthèse de lymphokine.
28. Procédé suivant l'une quelconque des revendications 19 à 27, caractérisé en ce que le produit chimique d'extraction de phase lipidique est un alcool à longue chaîne ou un éther halogéné.
- 25 29. Procédé suivant la revendication 28, caractérisé en ce que le produit chimique d'extraction de phase lipidique est du 2-octanol ou de l'isofluorane.

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